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Narrative review

Diagnosing infectious encephalitis: a narrative review

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ABSTRACT

Background: Diagnosing infectious encephalitis can be challenging as it can be caused by a wide range of pathogens, with viruses being the most common cause. In a substantial number of patients, no pathogen is identified despite a clinical diagnosis of infectious encephalitis. Recent advancements in diagnostic testing have introduced new methods to address this diagnostic challenge and improve pathogen detection.
Objectives: The objective of this study is to provide a comprehensive clinical approach for diagnosing infectious encephalitis and explore novel diagnostic methods.
Sources: We searched PubMed to identify relevant literature on diagnosing encephalitis in English up to 1 September 2024, as well as included articles known by the authors.
Content: Clinical characteristics may suggest a specific cause of infectious encephalitis, but are insufficient to guide treatment decisions. Therefore, cerebrospinal fluid (CSF) examination remains the cornerstone of the diagnostic process, with CSF leucocyte count being the most reliable predictor for central nervous system infections. CSF features can be normal, however, in a proportion of patients presenting with infectious encephalitis. A definite diagnosis of infectious encephalitis is established by microbiological or histopathological tests in ~50% of patients. Additional investigations, including neuroimaging or electroencephalography, can provide evidence for encephalitis or help to identify alternate conditions, although their role is primarily supportive. Emerging diagnostic techniques, including next-generation sequencing metagenomics and unbiased serology (Phage ImmunoPrecipitation Sequencing), have the potential to increase the proportion of patients with a confirmed diagnosis. However, these techniques are not yet practical because of their complex analysis, long turnaround times and high costs.
Implications: Microbiological confirmation is paramount in the diagnosis of infectious encephalitis, but it is currently established in about half of cases. Although novel techniques show promise to increase the proportion of cause-specific diagnoses, they are not yet suitable for routine use. This highlights the ongoing need for advancements in diagnostic methods. **Sabine E. Olie, Clin Microbiol Infect 2025;31:522**
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Introduction

Infectious encephalitis is a disease defined by inflammation of the brain parenchyma caused by the invasion of a pathogen [1]. Viruses are the most common cause, but bacteria, fungi and parasites are also frequent causes. The incidence and epidemiology of pathogens differ per geographical location and are dependent on a variety of factors, such as the season and contact with animals and insects. In addition, international travel, migration and climate change may result in shifting epidemiology [2,3]. Although a definite diagnosis of encephalitis is made through histopathological examination of brain tissue, the clinical diagnosis is generally based on a combination of clinical symptoms and ancillary investigations, such as quantitative PCR [4]. Among patients with a clinical diagnosis of suspected infectious encephalitis, current microbiological investigations are able to identify a pathogen in ~50% of patients [5,6]. This is probably because of factors such as varying assay sensitivity for different pathogens [7] and the potential presence of unknown pathogens. Multiplex PCR reduces the risk of not testing for known pathogens, albeit at the expense of sensitivity. Recent efforts to increase the rate of cause-specific diagnoses have shown promising results in research settings, but their applicability in clinical medical care is unclear.

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In this review, we provide a comprehensive clinical approach for the diagnosis of infectious encephalitis and explore recent advances in diagnostic methods. We searched PubMed to identify relevant literature until 1 September 2024.

Clinical features and diagnostic criteria

Encephalitis frequently presents with an altered mental status, fever, headache, seizures or focal neurological abnormalities [8]. However, bacterial meningitis, but also sepsis, metabolic disturbances and non-infectious neurological disorders may present with similar symptoms [9,10]. In 2013, the International Encephalitis Consortium proposed a standardized case definition of encephalitis, consisting of major and minor criteria, for use in epidemiology and research [11]. These research criteria consist of clinical symptoms and ancillary investigations and were based on literature review and expert opinion. Recently, these criteria have been validated in a prospective cohort of patients with a suspected central nervous system (CNS) infection where they showed high specificity, but limited sensitivity [6]. Criteria for clinical symptoms performed generally less well than those for biochemical analyses of cerebrospinal fluid (CSF) and magnetic resonance imaging (MRI) of the brain. Subsequent recalibration of the proposed criteria led to a substantial increase in sensitivity, albeit not enough to correctly include every case of encephalitis (Table 1) [6,11]. As such, clinical symptoms alone are insufficient to diagnose encephalitis. Yet, combining these symptoms with a patient's medical history, exposure to known risk factors and regional incidence and epidemiology can make the diagnosis more or less likely (Table 2 and Fig. 1) [6,11]. Important aspects to consider are recent illnesses, sexual history, the patient's immune and vaccination status, exposure to animals or insects and contact with animal products. The latter refers especially to the consumption of raw/partially cooked meat or unpasteurized milk as this increases the possibility of infections such as *Toxoplasma gondii* and *Listeria monocytogenes* [1]. In addition, a patient's travel history needs to be assessed as the prevalence of pathogens varies per geographical location [4]. For instance, whereas encephalitis caused by *Herpesviridae* occurs globally and is not season bound, infections caused by Japanese encephalitis virus are geographically restricted to Asian countries and are known to cause epidemics during summers and post-monsoon season [4,12]. It is important to consider that the epidemiology of infectious agents is dynamic and because of an increase in global travel and climate change, this especially impacts vector-borne pathogens such as *Flaviviridae* [13]. The rapid spread of West Nile virus (WNV) across the United States after an initial outbreak in New York in 1999 is a well-known example, and in recent years several outbreaks of WNV have occurred throughout the European continent [14–16].

In summary, clinical symptoms alone are not sufficient to diagnose encephalitis. However, a thorough assessment of a patient's medical history and risk factors in the context of their geographical location can guide further diagnostic investigations.

Table 1
Updated International Encephalitis Consortium encephalitis criteria with proposed cut-off values for possible and probable encephalitis[a]

Encephalitis criteria[b]	Updated weight		
Altered mental status, ≥24 h	2		
Fever, ≥38°C	0		
Seizures	3		
Focal neurological findings	0		
Elevated CSF leucocyte count, ≥5 cells/mm^3	5		
Abnormalities on neuroimaging	9		
Abnormalities on EEG	0		
Maximum score	19		
	Cut-off	Sensitivity	Specificity
Possible encephalitis	5	93% (95% CI 88–96%)	51% (95% 49–54%)
Probable encephalitis	8	51% (95% CI 44–59%)	91% (95% CI 89–92%)

CSF, cerebrospinal fluid.
[a] Modified from Staal et al., 2024 [6].
[b] Diagnostic criteria for encephalitis as proposed by the International Encephalitis Consortium [11]: an altered mental status lasting for 24 hours or more with no alternative cause identified; fever (≥38°C) within 72 hours before or after presentation; seizures not attributable to a pre-existing seizure disorder; new onset of focal neurological findings; elevated CSF leucocytes (≥5 cells per mm^3); abnormalities of brain parenchyma on neuroimaging suggestive of encephalitis that is either new from prior studies or appears acute in onset; abnormalities on electroencephalography (EEG) consistent with encephalitis and not attributable to another cause.

Conventional blood and CSF features

The diagnostic workup of patients suspected of encephalitis is focused on the examination of CSF rather than that of blood. Blood parameters for inflammation, such as C-reactive protein and leucocytes, generally have low predictive values for a CNS immune response [10,17]. CSF leucocytes offer the highest diagnostic accuracy for diagnosing CNS infections (area under the curve [AUC] = 0.93–0.94) and a leucocyte count above 2000 cells/μL is strongly indicative of bacterial meningitis (>99%) [10,17–19]. However, the predictive value for diagnosing a CNS infection decreases considerably in patients with lower leucocyte counts (AUC = 0.83 for CSF leucocytes between 5 and 1000 cells/μL), as this can also be seen in patients with other CNS disorders [17]. Infectious encephalitis frequently presents with moderately elevated CSF leucocytes and, as such, it can be difficult to distinguish from other CNS disorders [20]. In addition, a 2024 study found elevated CSF leucocyte count to be present in only 81% of infectious encephalitis cases [20]. CSF to blood glucose ratio can be diagnostic for bacterial meningitis but has a limited predictive value in differentiating infectious encephalitis from other causes of CNS inflammation. Slightly elevated CSF protein is common in infectious encephalitis, but also occurs in various other diseases and is therefore not specific [10]. A high CSF protein is indicative of bacterial meningitis (specificity of 99% for a concentration >2.2 g/L) [19], but also herpesvirus encephalitis can present with markedly elevated CSF protein [21,22]. Hence, the predictive value of CSF protein in determining the cause of infectious encephalitis is limited.

Microbiological investigation

A definite diagnosis of encephalitis is obtained through histopathological examination of the affected brain tissue, but this is rarely performed because of the invasive nature of a brain biopsy and the possibility of using CSF as a proxy in most cases. Currently, guidelines suggest that the role of a brain biopsy is limited in the initial evaluation of patient with suspected encephalitis, yet it can be considered in patients with encephalitis with an unknown aetiology who deteriorate during treatment [1,23–26]. Direct detection of a pathogen in the CSF of patients with suspected encephalitis serves as an alternative for confirming an infectious cause. One study found that PCR in CSF was positive in 53/54 (98%) biopsy-confirmed patients with herpes simplex virus type 1 (HSV-1) encephalitis, the most common sporadic cause of infectious encephalitis [27]. PCR is currently considered the reference standard for detecting viral DNA and RNA, but is also used for detecting bacteria, parasites and fungi, especially when cultures remain negative [8,28,29]. PCR is a relatively simple and quick method and

Table 2
Geographical location, risk factors and diagnostic evaluation of various pathogens causing encephalitis[a]

Pathogen	Geographical location	Specific risk factor(s)	Ancillary investigation
Bartonella bacilliformis	South America	Sandfly bite	- Antibody testing in blood and CSF - CSF and blood culture - CSF PCR
B. henselae	Worldwide	Contact with cats	- Antibody testing in blood and CSF - CSF culture - CSF and lymph node biopsy PCR
Borrelia burgdorferi	Europe, North America	Tick bite	- Antibody testing in blood and CSF - CSF PCR (low sensitivity)
Brucella spp	Africa, Middle East, southern Europe	Consumption of unpasteurized milk	- Antibody testing in blood and CSF - Blood PCR - Serum agglutination (titre >1:160)
Chikungunya virus	South America, Southeast Asia, China	Mosquito bite	- Antibody testing in blood and CSF
Coxiella burnetii	Worldwide	Contact with sheep and goats, consumption of raw meat	- Antibody testing in blood
Cryptococcus neoformans	Worldwide	Immunocompromised (HIV infection)	- CSF fungal culture - Cryptococcal antigen testing (CrAg) in CSF and blood - CSF India Ink staining - CSF PCR
Cytomegalovirus	Worldwide	Immunocompromised (HIV infection, transplant recipients), neonates	- CSF PCR
Dengue virus	(Central) America, South America, Southeast Asia, China, Australia	Mosquito bite	- Antibody testing in blood and CSF - Antigen (NS1) detection in blood or CSF
Eastern equine encephalitis virus	North, Central and South America	Mosquito bite	- Antibody testing in blood and CSF
Enteroviruses	Worldwide	Children, immunocompromised, unvaccinated (in case of poliovirus)	- CSF PCR - Throat or rectal swab PCR
Epstein–Barr virus[b]	Worldwide	Immunocompromised (HIV infection, transplant recipients), primary infection in adolescents	- CSF PCR - Antibody testing in blood and CSF
Herpes simplex virus type 1 and 2	Worldwide	—	- CSF PCR - Antibody testing in blood and CSF (incl. ratios)
Human herpes virus 6 and 7	Worldwide	Immunocompromised (haematopoietic cell transplant recipients)	- CSF PCR
HIV	Worldwide	Risk factors for HIV infection	- CSF and blood PCR
Human polyomavirus 2 (JC virus)	Worldwide	Immunocompromised (HIV infection, T-cell impaired immunity)	- CSF PCR - MRI for confluent white matter lesions
Japanese encephalitis virus	Southeast Asia, India, Nepal, China	Mosquito bite	- Antibody testing in blood and CSF
Leptospira spp	Worldwide	Freshwater exposure, contact with rats	- CSF PCR - Antibody testing in blood and CSF - Urine PCR
Listeria monocytogenes	Worldwide	Immunocompromised (pregnancy, glucocorticoid therapy, haematological malignancies), consumption of raw meat and unpasteurized dairy	- CSF and blood culture - CSF PCR
Measles virus	Worldwide	Unvaccinated	- Antibody testing in blood and CSF - CSF PCR - Nasopharynx and urine PCR
Mumps virus	Worldwide	Unvaccinated	- Antibody testing in blood and CSF - CSF or saliva PCR
Murray Valley encephalitis virus	Southeast Asia, China, Australia	Mosquito bite	- Antibody testing in blood and CSF
Mycobacterium tuberculosis	Worldwide, more common in developing countries	Immunocompromised (HIV infection)	- CSF culture - Ziehl-Neelsen staining CSF - CSF PCR
Nipah virus	Southeast Asia, China	Contact with pigs and bats	- Antibody testing in blood and CSF
Rabies virus	Central and South America, Africa, India, Nepal	Bite or scratches from infected mammals (e.g. dogs, bats, monkeys, foxes, and raccoons)	- Antibody testing in blood and CSF - CSF or saliva PCR
Rickettsia rickettsii	North, Central and South America	Tick bite	- Antibody testing in blood and CSF - PCR on skin biopsy in case of skin lesions
Rift Valley fever virus	Africa	Mosquito bite, contact with infected animal (can occur in cows, sheep, goats, camels)	- Antibody testing in blood - Blood PCR
Rubella virus	Worldwide	Unvaccinated	- Antibody testing in blood and CSF
St. Louis encephalitis virus	North, Central and South America	Mosquito bite	- Antibody testing in blood and CSF

Table 2 (*continued*)

Pathogen	Geographical location	Specific risk factor(s)	Ancillary investigation
Tick-borne encephalitis virus	Europe, Russia, Southeast Asia, China	Tick bite	- Antibody testing in blood and CSF
Toscana phlebovirus	Southern Europe	Sandfly bite	- Antibody testing in blood and CSF
Toxoplasma gondii	Worldwide	Immunocompromised (HIV infection), contact with cats, unpasteurized milk	- Antibody testing in blood and CSF - CSF PCR - MRI brain for typical lesions
Trypanosoma brucei spp.	Africa	Tsetse fly bite	- Direct microscopy - Antibody testing in blood and CSF
Usustu virus	Europe, Africa	Mosquito bite	- Antibody testing in blood and CSF
Varicella zoster virus	Worldwide	Immunocompromised (transplant recipients, immunosuppressive therapy)	- CSF PCR - Antibody testing in blood and CSF (incl. CSF to blood ratios) - PCR skin lesion
Venezuelan equine encephalitis virus	North, Central and South America	Mosquito bite	- Antibody testing in blood and CSF
West Nile virus	North and Central America, Europe, Africa, Middle East	Mosquito bite	- Antibody testing in blood and CSF
Western equine encephalitis virus	(Central) America, South America	Mosquito bite	- Antibody testing in blood and CSF
Zika virus	Southern hemisphere	Mosquito bite	- CSF PCR - Antibody testing in blood and CSF - Urine PCR

CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.
[a] Modified from Tunkel et al., 2008 [1].
[b] Central nervous system lymphoma and post-transplant lymphoproliferative disease should be considered if Epstein–Barr virus DNA is found in CSF.

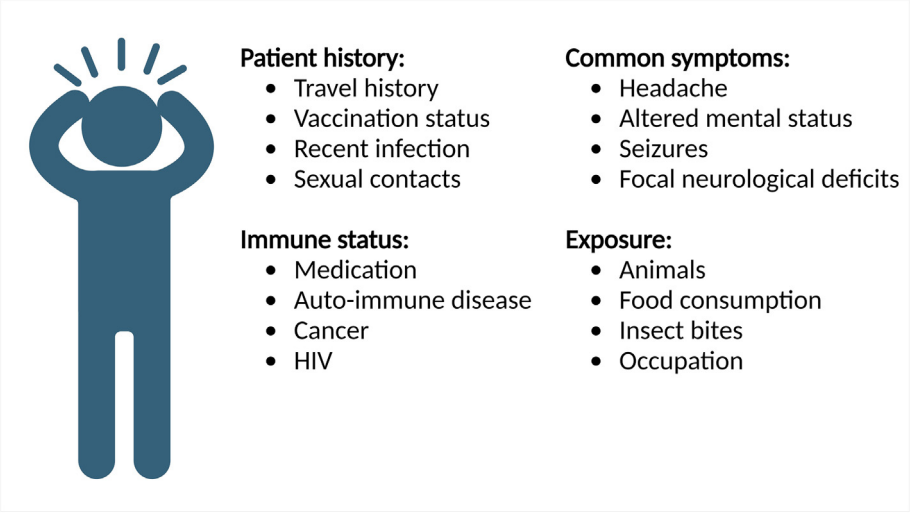

Fig. 1. Clinical symptoms, risk factors and medical history in suspected infectious encephalitis.

has the advantage that antimicrobial therapy initiated before the lumbar puncture only minimally influences its yield. However, the performance of PCR varies greatly per pathogen. Although for most viral pathogens the test characteristics are excellent, for other viruses PCR lacks accuracy. For instance, CSF PCR for HSV generally has a sensitivity and specificity of >95% [7]. However, HSV viral load might be low in the early phase of the disease, which may result in false-negative results [30,31]. Repeating HSV CSF PCR after 3–7 days is therefore recommended when HSV encephalitis is suspected and the first PCR is negative [32]. Under acyclovir treatment, PCR sensitivity decreases after ~7 days, prompting testing for specific antibodies in CSF [33]. In addition, there are several other factors that might influence PCR performance: some viruses, especially those from the *Flaviviridae* family, are thought to no longer be present in the CSF at the time of symptom onset and can therefore not always be detected by PCR at the time of the lumbar puncture [34]. In those cases, serological detection of virus-specific IgM antibodies can serve as a diagnostic proxy [35–37]. Other explanations for a lower accuracy of PCR are a low viral load in CSF or infections limited to the brain parenchyma in which the virus is not present in CSF. A major limitation of PCR is that it is pathogen specific, requiring a hypothesis concerning the causative pathogen before testing. Multiplex PCR panels are available but offer limited added value to singleplex (pathogen specific) assays. The yield of multiplex PCR panels depends strongly on the causative pathogen and can be challenging to interpret because of false-positive results [38]. This is most prominently the case for *Streptococcus pneumoniae*, where the number of false positives detected by the Filmarray Meningitis/Encephalitis panel (Biofire Diagnostics) is reported to be as high as 56% compared with CSF culture results [38]. In the context of diagnosis of infectious encephalitis, these false-positive results could lead to unnecessary or incorrect treatment. Furthermore, these assays often lack sensitivity for important encephalitis pathogens, for instance, for HSV-1, the Filmarray Meningitis/Encephalitis panel detected only 76% (95% CI: 51–90%) of PCR-confirmed cases [38]. Current guidelines recommend performing pathogen-specific PCR in the diagnostic workup of patients with suspected infectious encephalitis [1,23].

Although less common than viral pathogens, bacterial causes of encephalitis do occur, and the reference standard for diagnosis is CSF culture. However, because some bacterial pathogens are difficult to cultivate, such as *Borrelia burgdorferi* or *Treponema pallidum*, antibody testing serves as a proxy for direct detection of the bacterium.

Neuroimaging and other ancillary investigations

Neuroimaging, particularly MRI, plays an important role in the diagnostic workup of patients with suspected encephalitis as it can either be suggestive of a specific aetiology or identify alternate conditions. Computed tomography of the brain might show areas of oedema or haemorrhage prompting a cranial MRI, but generally does not show abnormalities specific for encephalitis and is usually normal [39]. Cranial computed tomography is primarily considered in emergency situations or when an MRI cannot be performed directly. Infectious encephalitis can present with a wide range of MRI abnormalities and specific patterns can be suggestive of an underlying aetiology. Different MRI sequences can further define, for instance, areas of oedema (T2-weighted imaging and fluid-attenuated inversion recovery [FLAIR]) or identify haemorrhages (susceptibility-weighted imaging [SWI]) and regions of active inflammation (post-gadolinium T1-weighted imaging).

Abnormalities on cranial MRI suggestive of HSV encephalitis are relatively well described and found in 81% (95% CI: 54–95%) of cases with a microbiologically confirmed diagnosis [39]. Frequent findings are FLAIR hyperintense lesion in the medial temporal lobe (Fig. 2) [40,41]. Bilateral, asymmetrical, involvement is considered highly indicative of HSV encephalitis, but usually occurs later on. Patients may also present with haemorrhage in the affected area. Temporal lobe FLAIR hyperintensities have also been described in other infectious and non-infectious aetiologies, such as *Mycobacterium tuberculosis* and acute autoimmune limbic encephalitis [42].

MRI abnormalities found in patients with encephalitis due to other infectious agents have low specificity. Hyperintensities on cranial FLAIR sequences of deep grey matter (basal ganglia and thalamus) or anterior horn cells in the spinal cord are suggestive of an infectious encephalo (-myelitis) caused by arboviruses [42]. Although Flaviviruses (e.g. WNV and tick-borne encephalitis virus) are important considerations, the MRI lesions are non-specific and imaging might even be normal. A prospective study of tick-borne encephalitis found that in patients with focal neurological symptoms, cerebral MRI abnormalities, if present, always involved the thalamus [43]. Involvement of the basal ganglia or thalamus on MRI was found in ≤5% of patients with neuroinvasive WNV infections, whereas brainstem involvement was observed in 36% [44]. Deep grey matter abnormalities on MRI also occur in other causes of infectious encephalitis [45] and various neurological diseases, such as focal vascular lesions or metabolic disturbances, and generally have low sensitivity. Although cranial MRI is the modality of choice for evaluating infectious encephalitis, a correct diagnosis can only be made by demonstrating the causative pathogen. Other imaging techniques, such as fluorodeoxyglucose (FDG) positron emission tomography, are primarily used for the detection of concomitant malignancies or autoimmune disease in patients with suspected autoimmune or paraneoplastic encephalitis.

Electroencephalography (EEG) is frequently used in clinical care of patients with suspected encephalitis but rarely produces pathognomonic findings. EEG is the cornerstone test to distinguish encephalopathy from a non-convulsive status epilepticus and focal abnormalities on EEG are highly correlated with focal abnormalities on a cranial MRI. In addition, when interpreted within the context of suspected encephalitis, EEG abnormalities might be suggestive of a specific pathophysiology [46]. HSV-1 might show lateralized periodic discharges, whereas an extreme delta brush is strongly associated with anti-N-methyl-D-aspartate (NMDA) receptor encephalitis [46]. Although not conclusive, these findings prompt additional assessment (e.g. neuroimaging, detection of autoimmune antibodies and/or microbiological testing) to establish a definitive diagnosis.

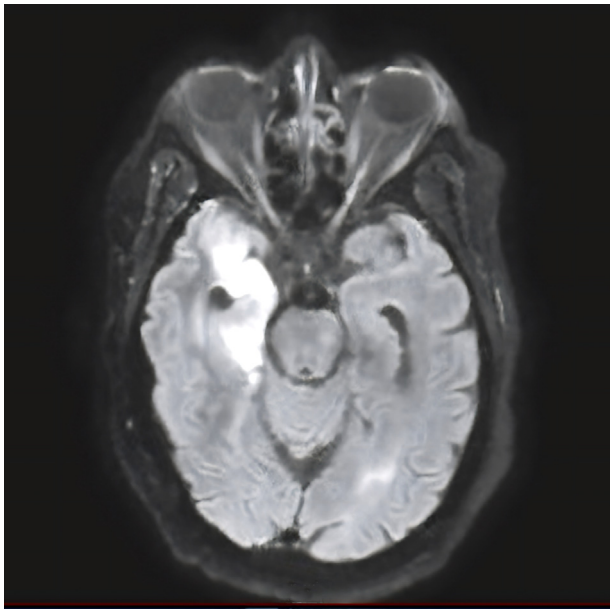

Fig. 2. Magnetic resonance imaging fluid-attenuated inversion recovery enhancement of the medial temporal lobe in herpes simplex virus type 1 encephalitis.

Novel diagnostics and future perspectives

A promising novel method for diagnosing infectious encephalitis is metagenomic next-generation sequencing (mNGS). This technique holds the potential to detect all pathogenic DNA or RNA present in a patient sample by sequencing all genetic material and therefore provides an unbiased approach to finding a causative pathogen. Previous studies have investigated the performance of mNGS for the diagnosis of a CNS infection. A meta-analysis from 2022 included 12 studies with a total of 1249 patients and showed a pooled sensitivity of 77% (95% CI: 70–82%) and a pooled specificity of 96% (95% CI: 93–98%) for all pathogens [47]. Performance was better for viral pathogens, with a pooled sensitivity of 82% (95% CI: 75–90%). Although mNGS shows promise as a method to diagnose infectious encephalitis, there are several important limitations. Some pathogens might not be detected because of low amount of RNA or DNA in the CSF, which leads to false-negative results [48,49]. A solution for low input of genetic material might be target-enriched metagenomics. In this variation of mNGS, an enrichment step is added to the procedure, allowing specific DNA sequences of the targeted pathogens to be amplified and thereby increasing sensitivity. One study comparing regular ‘shotgun’ metagenomics to target-enriched metagenomics found an increase in sensitivity from 44% to 84% of the mNGS in 89 patients with a confirmed CNS infection [50]. Even with a targeted approach, the complex bioinformatic analysis, long turnaround time and high costs make mNGS currently not suitable to be used in routine clinical practice [51]. mNGS might aid in establishing a cause-specific diagnosis in patients with encephalitis where routine microbiological examination proved insufficient.

'Phage ImmunoPrecipitation Sequencing,' or PhIP-Seq, offers a different approach for the identification of pathogens in viral encephalitis. Utilizing bacteriophages and patient serum from two consecutive time points, this technique is able to detect antibodies against a specific virus and retrospectively determine the causative pathogen [52]. Bacteriophages are viruses that target bacteria and can be utilized to express certain viral proteins on their surface, by introducing specific viral DNA fragments into their genomes. When performing this process for over 1000 viruses, this results in a collection of bacteriophages expressing a wide range of viral proteins. Patient serum is then added to this collection, and antibodies present in the serum bind to virus-specific bacteriophages. By comparing patient serum from the acute phase of the disease to serum from 6 to 8 weeks after disease onset, the potential pathogen can be identified. Viruses that were previously not associated with encephalitis can subsequently be included in regular diagnostic through specific PCR assays. A study conducted in 36 patients with viral encephalitis showed an increase of cause-specific diagnosis from 9 to 13 patients (44%) by using PhIP-Seq [53]. Phip-Seq shows the potential to discover novel viral causes of encephalitis; however, current evidence is limited and more research is needed to thoroughly assess clinical applicability.

Conclusion

The diagnosis of infectious encephalitis can pose a challenge for physicians. Clinical symptoms and ancillary investigations are non-specific and diagnostic criteria lack sensitivity to be used as a stand-alone tool in clinical practice. Direct detection of a pathogen is the reference standard, but a large proportion of patients with suspected infectious encephalitis do not obtain microbiological confirmation. Although new diagnostic methods are developed and may offer future solutions, they are currently unsuitable for routine clinical use because of their complex analysis and interpretation, and high costs.

Author contributions

SEO and SLS were responsible for conceptualization, methodology, investigation, and writing – original draft. DvdB was responsible for writing – review and editing. MCB was responsible for conceptualization, methodology, writing – review and editing, supervision, project administration and funding acquisition. All authors read and approved the final manuscript.

Transparency declaration

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